REMARKS/ARGUMENTS:

Claims 32, 34-36, 41, 55, 58-60, 64, and 69 are amended. Support for the amendments to claims 32, 41, 55, 64 and 69 can be found, e.g., at p. 3, lines 7-10 and p. 16, lines 14-17 of Applicant's specification. Support for the amendments to claims 34-36 and 58-60 can be found, e.g., in the Examples 5, 7-11 and at p. 15, line 22-p. 19, line 8 of Applicant's specification. Claims 32, 34-36, 38, 40-45, 55, 58-61, and 63-71 are pending in the application. Reexamination and reconsideration of the application, as amended, are respectfully requested.

The present invention relates generally to gene silencing phenomenon, and particularly to gene silencing using mRNA-cDNA hybrids and methods for generating mRNA-cDNA hybrids fro use in gene silencing. (Applicant's specification, at p. 1, lines 6-8).

CLAIM REJECTION UNDER 35 U.S.C. § 112, 1ST PARAGRAPH:

Claims 32, 34-36, 38, 40-45, 55, 58-61, and 63-71 stand rejected under 35 U.S.C. § 112, first paragraph, because the specification, while being enabling for a method of inhibiting β-catenin expression *in vivo* in selected organs of chicken embryos using a mRNA-cDNA hybrid duplex, does not reasonably provide enablement for a method of inhibiting expression from any target gene using a mRNA-cDNA hybrid duplex. The Applicant respectfully traverses this rejection.

The Office cites studies from Parrish and Tuschl to demonstrate the unpredictability in the art associated with the inhibition of gene expression using RNA-DNA hybrid duplexes.

Applicant respectfully disagrees. The Applicant's specification, particularly Examples 5 and 7-11 provide sufficient support, guidance, and enablement for a method of inhibiting targeted gene expression using long mRNA-cDNA hybrid

duplexes *in vitro* and *in vivo*. However, in order to expedite the prosecution of the instant application, Applicant amended independent claims 32 and 69 to clarify (i) that the gene silencing mechanism of the mRNA-cDNA hybrid duplex goes through the intracellular RNA interference (RNAi) mechanism, wherein the expression is inhibited through the intracellular RNA interference mechanism, and (ii) that the length of the mRNA-cDNA hybrid duplex is more than 500 base pairs.

As discussed in Parrish and Tuschl, small double-stranded RNA (siRNA) molecules are capable of triggering gene silencing in mammalian cells. The siRNAs tested in Parrish and Tuschl were approximately 19–25 nucleotide base pairs. In this size range, Parrish and Tuschl teach the unpredictability of the use of short RNA-DNA hybrid duplexes (19–25 base pairs) in triggering RNAi-related gene silencing. However, neither Parrish nor Tuschl teach or suggest the present invention, which is the use of long RNA-DNA hybrid duplexes that are larger than 500 base pairs. In view of these totally different standards, the findings of Parrish and Tuschl are irrelevant to the enablement of the present invention.

Furthermore, recent studies in RNA-DNA duplex-mediated gene silencing have demonstrated that the D-RNAi mechanism of the present invention relies on the generation of small intronic microRNA, or recently named piRNA, rather than siRNA. This gene silencing mechanism functions through a coupled interaction between Pol-II-directed pre-mRNA transcription and RNA splicing excision, occurring proximal to the genomic perichromatin fibrils (Lin and Ying (2001), Exhibit A; Lin and Ying (2004), Exhibit B; Bateman and Wu (2007), Exhibit C), indicating that the mRNA–perichromatin DNA interaction facilitates the generation of new small gene silencing RNAs by a certain intracellular RNA-dependent RNA polymerase (RdRp). Mammalian type-II RNA polymerases (Pol-II) have been observed to possess this RdRp activity (Lin et al. (2001), Exhibit D;

Filipovska and Konarska (2000), Exhibit E; Modahl et al. (2000), Exhibit F). Because the smallest mRNAs naturally transcribed by Pol-II are approximately 300 base pairs, the mRNA–perichromatin DNA duplex template for triggering the D-RNAi mechanism must be larger than 300 base pairs, more preferably larger than 500 base pairs. The small intronic microRNA/piRNA molecules so obtained are single-stranded RNAs capable of modulating targeted gene expression through an intracellular gene silencing mechanism, termed RNA interference (RNAi). Several recent studies have also demonstrated that the mRNA–perichromatin DNA interaction results in the generation of Piwi-interacting RNAs (piRNA), which are distinct from other small double-stranded siRNAs and shRNAs by their relatively larger size, single-strandedness, and strand-specificity (Bateman and Wu (2007), Exhibit C; O'Donnell and Boeke (2007), Exhibit G). As shown in Figure 2 of Lin and Ying (2001), Exhibit A and Figure 2 of O'Donnell and Boeke (2007), Exhibit G, the piRNA class of small RNAs is transcribed by Pol-II, as a RdRp, from the mRNA–perichromatin DNA duplex region of a replicating cell genome during mitosis or meiosis. Nuclear transfection of long DNA-RNA duplex templates as described in the present invention has also been shown to trigger intronic microRNA/piRNA-like gene silencing effects against viral infection and retrotransposon activity (Lin and Ying (2001), Exhibit A; Lin and Ying (2004), Exhibit B). In *Drosophila* and zebrafish, Piwi proteins have been recently found to be implicated in piRNA biogenesis to maintain transposon silencing in the germline genome (O'Donnell and Boeke (2007), Exhibit G). This function may be conserved in mice as loss of Miwi2, a mouse Piwi homolog, leads to germline stem cell and meiotic defects correlated with increased transposon activity (Carmell et al. (2007), Exhibit H). Because the RNAi effector of the piRNA-mediated gene silencing requires Piwi proteins rather than siRNA/shRNA-associated RNase-III Dicers, this

suggests that the intronic microRNA/piRNA-mediated RNAi mechanism is different than the siRNA/shRNA-mediated RNAi pathway (O'Donnell and Boeke (2007), Exhibit G).

In view of the known D-RNAi mechanism, discussed above, and the use of long RNA-DNA duplex templates in the present invention, the unpredictability in Parrish and Tuschl is rendered moot. Furthermore, Examples 5 and 7-11 in the Applicant's specification provide sufficient support, guidance, and enablement for a method of inhibiting targeted gene expression using long mRNA-cDNA hybrid duplexes *in vitro* and *in vivo*.

In view of the foregoing, it is respectfully submitted that the application is in condition for allowance. Reexamination and reconsideration of the application, as amended, are requested.

If for any reason the Examiner finds the application other than in condition for allowance, the Examiner is requested to call the undersigned attorney at the Los Angeles, California telephone number (310) 785-4600 to discuss the steps necessary for placing the application in condition for allowance.

If there are any fees due in connection with the filing of this response, please charge the fees to our Deposit Account No. 50-1314.

Respectfully submitted,
HOGAN & HARTSON L.L.P.

Date: May 14, 2007

By: 
Barry M. Shuman
Registration No. 50,220

1999 Avenue of the Stars, Suite 1400
Los Angeles, California 90067
Phone: 310-785-4600
Fax: 310-785-4601